ELSEVIER

Biophysical Chemistry 132 (2008) 18–22

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

Crystallization of metastable β glycine from gas phase via the sublimation of α or γ form in vacuum

Zhimin Liu [a,b], Lin Zhong [a], Pinliang Ying [a], Zhaochi Feng [a], Can Li [a,*]

[a] State Key Laboratory of Catalysis, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, P.O. Box 110, Dalian 116023, China
[b] Graduate School of the Chinese Academy of Sciences, China

Received 8 August 2007; received in revised form 30 September 2007; accepted 1 October 2007
Available online 13 October 2007

Abstract

It is found that β glycine, the metastable polymorph of glycine, can be rapidly formed from gas phase via the sublimation of its stable α or γ form in vacuum. The transformation process was monitored by infrared spectroscopy and the crystal structure of the sublimate was identified by X-ray diffraction techniques. It is the first report about the transformation of stable α or γ glycine into metastable β form in "one-step" (heating then cool down spontaneously). Crystallization of β glycine from gas phase is very different from other methods that require additives in solution. The hydrogen-bonding interaction and self-assembling of amino acid were discussed based on the observations.

Keywords: Crystallization; Gas phase; Glycine; Polymorphism; β Glycine; Sublimation

1. Introduction

Polymorphism, the phenomenon of a chemical species having more than one possible crystal form, has been intensely studied, yet remains poorly understood and controlled [1–3]. Polymorphs can exhibit different mechanical, thermal, and physical properties, such as compressibility, melting point, solubility, and crystal habit, which can have a great influence on the bioavailability, filtration, and tableting processes of pharmaceutical, food, and specialty materials [4–6]. The polymorphism also exists in the crystal structures of amino acids with different packing arrangements and hydrogen-bonding patterns of the zwitterions, as reported for glycine and L-glutamic acid [6]. The amino acids play a major role in the life of organisms on this planet, and as such have commanded our interest and attention. To some extent they represent a model system for the investigations of protein folding and crystallization, which are strongly controlled by hydrogen-bond interactions [6–9].

Glycine ($^{+}H_3NCH_2COO^{-}$) is the simplest one among the amino acids and is quite abundant in various proteins and enzymes. The characterization of its crystal forms is useful for studying the properties of amino acids and hydrogen bonds in biological systems [6–9].Glycine exists in three polymorphic forms: α, β, and γ [10–15]. Transformations between α, β, and γ polymorphs have been studied [12–15]. The γ form transforms to the α form on heating to around 170 °C [12]. The α-to-γ transformation occurs at high humidity [13]. The β form transforms rapidly to α or γ form in the presence of moisture at room temperature, but it is metastable in dry air [14,15]. The ability to control the transformation and crystallization process is critical in order to ensure that the correct polymorph is produced. The β glycine crystals can be obtained from ethanol–water mixed solution of glycine or octanoic acid-in-water emulsions, but both the methods need careful operation [14,16]. However, there is not any report about the transformation of α or γ glycine into metastable β form. In this work, we found that β glycine can be formed from gas phase via the sublimation of α or γ glycine in vacuum.

* Corresponding author. Tel.: +86 411 84379070; fax: +86 411 84694447.
E-mail address: canli@dicp.ac.cn (C. Li).
URL: http://www.canli.dicp.ac.cn (C. Li).

0301-4622/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2007.10.003

2. Materials and methods

2.1. Chemicals

The glycine and CH_3OH were commercial preparation from Sigma-Aldrich. The H_2O was doubly distilled. H_2O and CH_3OH were both further purified by freeze–thaw cycles in the vacuum system.

2.2. Apparatus and methods

The crystallization of β glycine from gas phase and polymorphic transformation were studied using Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) techniques. In this work, glycine was sublimated rapidly in vacuum and deposited spontaneously onto a CaF_2 wafer in a quartz IR cell (the diagram of the IR cell is shown in Supplementary material Fig. S1). The quartz cell used has a 15 mm gap distance between the CaF_2 wafer and the bottom where the amino acids were placed and heated for sublimation. The IR cell was vacuumed by a pump (to 0.1 Pa) for 30 min and then closed before sublimation.

All the IR spectra were collected on a FTIR spectrometer (Nicolet Nexus 470) with a resolution of 4 cm^{-1} and 64 scans in the region of 4000–1000 cm^{-1}. The powder X-ray diffraction patterns were collected on an X-ray diffractometer (Rigaku D/Max2500VB2). The elemental analysis was done on a CHN element analyzer (Perkin-Elmer 2400 Π). The weight percentage of oxygen is obtained by: O%=100%−(C%+H%+N%).

3. Results

3.1. In-situ IR spectra of the sublimation of glycine

The IR spectrum of starting glycine is different from that of the sublimate as shown in Fig. 1. First, the weak band at about 3107 cm^{-1}, assigned to the antisymmetric stretching of the NH_3^+ group, shifts to 3182 cm^{-1}. Second, the asymmetric stretching (υ_{as}) of COO^- and the asymmetric bending (β_{as}) of NH_3^+ are

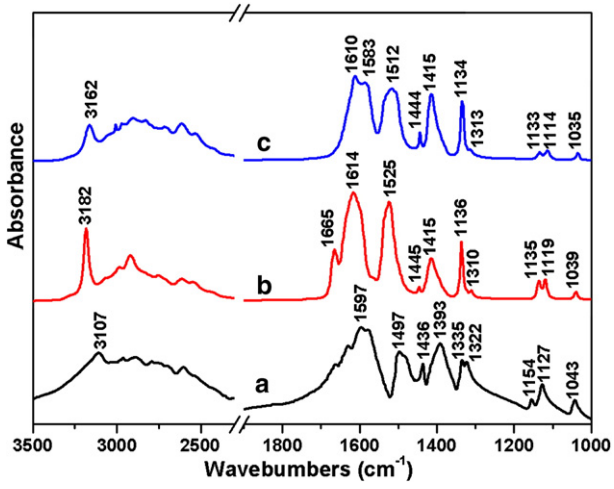

Fig. 1. The IR spectra of glycine: (a) KBr pellet in air, (b) the sublimate in vacuum, (c) the sublimate exposed to moisture for 30 min.

Table 1
Elemental analysis of the sublimate of glycine

Sample names	Methods	Elemental analysis (wt.%)			
		C	H	N	O
$(CH_2CONH)_n$	Calculation	42.10	5.30	24.55	28.04
Glycly-glycine	Calculation	36.36	6.10	21.20	36.33
Glycine	Calculation	32.00	6.71	18.66	42.63
Sublimate of glycine	Experiment[a]	32.09	6.62	19.22	42.07

The calculated values of glycine and the peptides of glycine are shown for comparison.

[a] The result was obtained on the CHN element analyzer. The weight percentage of oxygen is obtained by: O%=100%−(C%+H%+N%).

overlapped at ca. 1597 cm^{-1} in Fig. 1a and this band splits and shifts to higher frequencies at 1665 and 1614 cm^{-1} for the sublimate. Other bands also move toward higher wavenumbers: the symmetric bending (β_s) of NH_3^+ (1497 to 1525 cm^{-1}), the symmetric stretching (υ_s) of COO^- (1393 to 1415 cm^{-1}) and the bending of CH_2 (1436 to 1445 cm^{-1}). It is clear that transformations occur in the process of sublimation and cooling.

After exposed to dry air, the sublimate can maintain its IR characteristics for several days as it is in vacuum. But if the sublimate meets moisture or is ground with KBr in air, its IR spectrum turns into that of Fig. 1c, which is very different from the spectrum of Fig. 1b. The IR band at about 3182 cm^{-1} shifts to 3162 cm^{-1}. The band at 1665 cm^{-1} disappears, while a shoulder at 1583 cm^{-1} emerges. The band at 1525 cm^{-1} shifts to lower frequency at 1512 cm^{-1}.

Gross and Grodsky have shown that glycine can sublimate in vacuum without decomposition and condensation [17]. CO_2, NH_3 or H_2O, which may be the products of glycine decomposition, are not detected by IR spectroscopy in our experiments. It was reported that during a cyclic heating/drying/wetting process of glycine solutions in the presence of oxides, some oligopeptides, such as glycyl-glycine, the cyclic dimer diketopiperazine, and triglycine, are produced on silica and alumina [18,19]. Generally these oligopeptides, with IR characteristics of $\upsilon_{C=O}$ in the range of 1650–1800 cm^{-1}, are formed very slowly [18,19]. In this work, the sublimate (white solid) was obtained in several minutes by rapid heating, so the band at 1665 cm^{-1} in Fig. 1b could not be due to the oligopeptides. Spectroscopic and theoretical studies have shown that the glycine exists as the neutral forms in the vapor phase [20,21]. However, it is clear that the neutral forms of glycine molecules are not present in the solid of sublimate as the absence of absorbance in the range of 1700–1800 cm^{-1} ($\upsilon_{C=O}$ of the COOH group) in Fig. 1b.

If peptides were formed in the sublimation process of glycine, the percentages of elements would change remarkably. But the elemental composition of sublimate is consistent with the calculated values of glycine, as the elemental analysis result given in Table 1. This result indicates that decomposition and condensation do not occur in the sublimation process. The sublimate is still glycine, but it exists as a different form from that in the original glycine.

In all the three spectra of glycine there are broad intense bands from 2200 to 3300 cm^{-1}. Generally, this absorption is

due to the N–H stretching of NH_3^+ hydrogen bonded with COO^- in the crystal lattice of amino acid [22,23]. The glycine molecules exist as the zwitterions with different hydrogen-bonding networks in the three types of solids, as indicated by the IR spectra. Compared with the spectroscopic studies of different glycine crystals in literature, it is found that the IR spectra in Fig. 1a, b and c are almost the same as those of γ, β and α polymorphs respectively [15,24–26]. Also the conditions needed for the transformation of Fig. 1b to c are similar to those of β to α glycine [12,15].

3.2. XRD of the sublimate of glycine

To verify whether the sublimate of glycine exists in an ordered structure or amphorous state and whether the three solids of glycine are γ, β and α polymorphs respectively, they were examined by powder X-ray diffraction, as shown in Fig. 2. The starting glycine is in the γ polymorph as the peak at 25.5°, corresponding to the (110) reflection of the γ polymorph, is observed (Fig. 2a) [10]. The sublimate of glycine coagulates as a film on glass plate was measured by XRD quickly after it was exposed to the air (Fig. 2b). A prominent peak at ~18° was observed, which was assigned to the (001) reflection of the β polymorph [14,15]. Once the sublimate was exposed to moisture or slightly ground in air, a set of peaks would appear, which is assigned to the reflections of the α form (Fig. 2c and d) [11,15]. It is clear that the XRD results are consistent with the IR spectra.

After the sublimation process in vacuum, the residual glycine that does not sublimate was also examined by XRD (not shown). It was found that the starting glycine (γ form) has transformed to the α form by heating in the sublimation process. Though glycine can sublimate above 118 °C, the sublimation is slow even up to 150 °C [17]. To get a fast sublimation, higher temperature is required. Once the temperature is above 170 °C [12], the γ form glycine transforms to the α form before sublimation. Using α form glycine as the starting material, the similar result was obtained: β glycine can be formed via the sublimation of the stable α polymorph. After the sublimation of γ glycine, the sublimate (β or mixture of β and α glycine) was gathered and sublimated in vacuum again. IR and XRD results similar to Figs. 1b and 2b were obtained (not shown), which corroborate that glycine does not decompose in the sublimation process in vacuum.

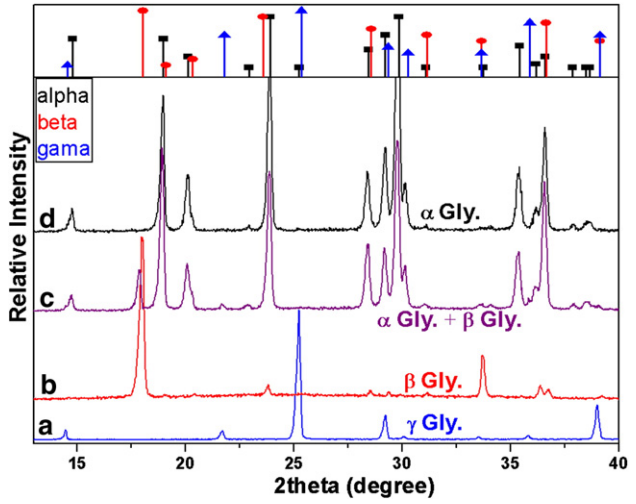

Fig. 2. The XRD patterns of glycine: (a) the starting glycine material, (b) the sublimate of glycine on glass plate, (c) the sublimate ground slightly and (d) the sublimate exposed to moisture. The top shows the standard XRD patterns of glycine: α (■), β (●) and γ (▲).

3.3. Sublimation in the presence of other molecules

Many factors, such as pH values, solvents and additives, can affect the crystallization of glycine in solutions and result in different polymorphs by controlling the conditions of crystallization [10,11,14]. The sublimation of glycine in the presence of air, H_2O and CH_3OH was investigated in order to study the effect of the surrounding conditions on glycine crystallization from gas phase. Heating in air (ambient condition) or CH_3OH vapor (100 mm Hg) the decomposition of glycine occurs to result in the extensive darkening of the material and generate NH_3 as shown by IR spectra (in Supplementary material Fig. S2). The oxidation of glycine also occurs in air as CO_2 observed by IR. The mixture of α and β glycine is obtained and the residual glycine becomes slightly yellow when subjected to the sublimation procedure in the presence of H_2O vapor. Crystallization of glycine from gas phase is also sensitive to the surrounding conditions. The interactions between glycine and other molecules make against the crystallization of β glycine from gas phase via the sublimation.

4. Discussion

The configuration states of glycine zwitterions in the three polymorphs differ from one another only by the angle between C–N and the least-squares plane (a twist around the C–C bond): 12.8° in γ-, 18.6° in α-, 24.8° in β-phases [12]. The three forms of glycine are the consequences of the complicated packing of molecules in the crystal lattice and the competition of several kinds of interactions: a) van der Waals, b) electrostatic and c) framework hydrogen bonding. The network of hydrogen bonding plays an essential role in the organization of the crystal structure of glycine.

The ability to control crystal polymorphism is of paramount importance in physical chemistry, pharmacology, and material sciences. However, the conditions to induce the precipitation of various (especially of metastable) polymorphs are invariably achieved by “mix and try” methods, which are kinetically driven. The three polymorphs of glycine can be formed under different solution conditions. α glycine is formed by spontaneous nucleation of pure aqueous glycine [11], while γ glycine is formed from aqueous acetic acid or ammonia solution [10]. β glycine can be formed by precipitation from ethanol–water mixtures [14]. Although the thermodynamic stability and density of the three polymorphs of glycine at room temperature are both in the order γ>α>β [12], they can be crystallized at different conditions respectively, indicating that the spontaneous nucleation of glycine is kinetically rather than thermodynamically controlled [29].

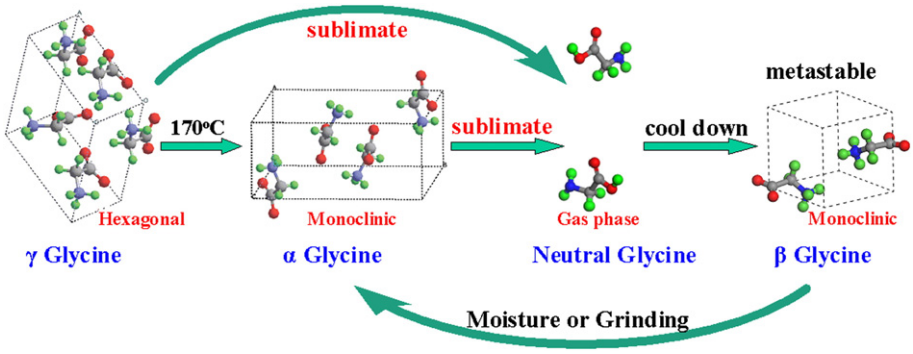

Fig. 3. The transformations of glycine during sublimation process.

For the crystallization from solution, a comprehensive theory of nucleation does not exist at present, but there is growing evidence that it is a two-step process: the formation of liquidlike clusters of solute molecules, followed by the rate-limiting organization of such a cluster into a protocrystal [30]. Various factors may play roles in these complex processes, such as, the formation of structured clusters prior to crystallization, the structure of growing surfaces that delineates emerging nuclei, the interaction between these surfaces and the solvent, as well as solvent–solute and solute–solute interactions [29]. The crystal structure of β glycine consists of hydrogen-bonded monomer units, as different from that of α glycine which comprises cyclic hydrogen-bonded pairs [11,14]. The addition of alcohol into water reduces the solubility of glycine. This reduced solubility should result in an increased concentration of solvated glycine monomers relative to that of hydrogen-bonded cyclic dimers, which will prefer the precipitation of β glycine from alcohol–water solutions [29].

Crystallization from gas phase or solution makes glycine molecules from disordered (relative free) states into ordered packing arrangements. The thermodynamically more stable α- and γ- polymorphs of glycine do not precipitate in the sublimation crystallization, indicating that crystallization from gas phase is also kinetically controlled. Compared with the crystallization from solution, crystallization by sublimation is a solvent-free process. The glycine–glycine interactions (hydrogen bonding, van der Waals and electrostatic) dominate the nucleation of glycine from gas phase, rather than the glycine–solvent interactions play an important role in crystallization from solution. As indicated by the IR band frequencies of N–H stretching in the three polymorphs of glycine, β form has the weakest hydrogen-bond networks. The metastable β glycine formed from sublimation crystallization is ascribed to rapid cooling of the hot vapor on the cold substrate, conditions under which hydrogen-bonded clusters are likely to condense in a crystalline arrangement with low density and weak hydrogen bonds.

It is well known that the proton transfer leading to the zwitterion → neutral glycine tautomerization occurs during sublimation. In the gas phase, the neutral glycine exists in a disordered state (with the highest free energy) and the interactions between glycine molecules are very weak [20,21]. When the hot glycine molecules land on the cooled substrate surface the thermal energy can be used to promote the proton transfer, converting the neutral species into the most stable zwitterions (in the solid). According to Ostwald's "Rule of Stages" [27,28], the metastable form should appear first during the crystallization from solution and then it should transform into the stable form. Though the crystallization of glycine is performed from gas phase in this study, neutral glycine (an unstable state at room temperature) does not directly become the most stable α or γ form (with the lowest free energy), but prefers to form the metastable β polymorph (intermediate stages) having the closest free energy to the neutral state. The "Ostwald's rule" still works even if the solvent is absent. Based on our results and the above discussion, the transformations involved in the formation of β glycine in vacuum are described in Fig. 3.

The crystallization and polymorphism of glycine, the simplest amino acid and the prototype structural unit of other biologically important amino acids and proteins, have been the subject of numerous studies. The study of glycine crystallization by sublimation can offer the information about the kinetics of hydrogen-bonding interaction and self-assembling between amino acids without the influence of solvent, which is difficult to clarify from the general experiments in solution. The hydrogen-bonding interactions between amino acid units play an important role in the protein folding and interactions [31,32]. Crystallization of protein in water is usually used to study the structure and folding of protein. Generally 40–60% water is present in the crystal of protein, which also can contribute to the protein folding and interaction with other bio-substance. It is important in science to differentiate the interactions between amino acid units in protein from those between protein and water [33]. To some extent, the crystallization of glycine by sublimation represents a model system for the investigations of pure interactions between amino acid units in protein and enzymes.

5. Conclusion

Our findings reveal that the metastable β glycine can be crystallized from gas phase via the sublimation of α, β or γ form in vacuum. It is the first report about the transformation of stable α or γ glycine into metastable β form in "one-step" (heating then cool down spontaneously). This approach is very different from other methods that require additives in solution. Crystallization of glycine from gas phase is kinetically rather

than thermodynamically controlled. The "Ostwald's rule" seems to still work even if the solvent is absent in the crystallization by sublimation.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (NSFC, Grant Nos. 20621063 and 20590363).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bpc.2007.10.003.

References

[1] J. Bernstein, Polymorphism in Molecular Crystals, Clarendon, Oxford, 2002, Chapter 10.
[2] R.J. Davey, Pizzas, polymorphs and pills, Chem. Commun. 13 (2003) 1463–1467.
[3] J.D. Dunitz, J. Bernstein, Disappearing polymorphs, Acc. Chem. Res. 28 (1995) 193–200.
[4] L. Yu, S.R. Byrn, et al., Thermochemistry and conformational polymorphism of a hexamorphic crystal system, J. Am. Chem. Soc. 122 (2000) 585–591.
[5] A.D. Bond, R. Boese, G.R. Desiraju, On the polymorphism of aspirin, Angew. Chem., Int. Ed. 46 (2007) 615–617.
[6] Boris Schade, Jürgen-Hinrich Fuhrhop, Amino acid networks, New J. Chem. 22 (1998) 97–104.
[7] M.I. Abd-Elrahman, et al., Kinetics of solid state stability of glycine derivatives as a model for peptides using differential scanning calorimetry, Biophys. Chemist. 97 (2002) 113–120.
[8] J. Huang, W.Y. Sun, W.X. Tang, et al., The interaction of glycine, aspartic acid, and lysine by the protonated macrocyclic ligand 6,19-bis(2-hydroxypropyl)-3,6,9,16,19,22-hexaaza-tricyclo-[22.2.2.211,14]triaconta-11,13,24,26,27,29-hexaene, Bioorg. Med. Chem. 12 (2004) 529–535.
[9] R. Prei ßner, P. Bork, On α-helices terminated by glycine: 1. Identification of common structural features, Biochem. Biophys. Res. Commun. 180 (1991) 660–665.
[10] Y. Iitaka, The crystal structure of γ-glycine, Acta Crystallogr. 14 (1961) 1–10.
[11] G. Albrecht, R.B. Corey, The crystal structure of glycine, J. Am. Chem. Soc. 61 (1939) 1087–1103.
[12] G.L. Pervolich, L.K. Hansen, A. Bauer-Brandl, The polymorphism of glycine. Thermochemical and structural aspects, J. Therm. Anal. Calorim. 66 (2001) 699–715.
[13] H. Sakai, H. Hosogai, et al., Transformation of α-glycine to γ-glycine, J. Cryst. Growth 116 (1992) 421–426.
[14] Y. Iitaka, The crystal structure of β-glycine, Acta Crystallogr. 13 (1960) 35–45.
[15] E.S. Ferrari, R.J. Davey, et al., Crystallization in polymorphic systems: the solution-mediated transformation of β to α glycine, Cryst. Growth Des. 3 (2003) 53–60.
[16] C.E. Nicholson, S.J. Cooper, M.J. Jamieson, Unique crystal morphologies of glycine grown from octanoic acid-in-water emulsions, J. Am. Chem. Soc. 128 (2006) 7718–7719.
[17] D. Gross, G. Grodsky, On the sublimation of amino acids and peptides, J. Am. Chem. Soc. 77 (1955) 1678–1680.
[18] M. Meng, L. Stievano, J.F. Lambert, Adsorption and thermal condensation mechanisms of amino acids on oxide supports. 1. Glycine on silica, Langmuir 20 (2004) 914–923.
[19] V.A. Basiuk, T.Y. Gromovoy, A.M. Glukhoy, V.G. Golovaty, Chemical transformations of proteinogenic amino acids during their sublimation in the presence of silica, Orig. Life Evol. Biosph. 21 (1991) 129–144.
[20] J.H. Jensen, M.S. Gordon, On the number of water molecules necessary to stabilize the glycine zwitterion, J. Am. Chem. Soc. 117 (1995) 8159–8170.
[21] A.G. Zavagliaab, R. Fausto, Low-temperature solid-state FTIR study of glycine, sarcosine and *N*,*N*-dimethylglycine: observation of neutral forms of simple amino acids in the solid state, Phys. Chem. Chem. Phys. 5 (2003) 3154–3161.
[22] A. Pawlukojc, J. Leciejewicz, et al., Neutron scattering, infrared, Raman spectroscopy and ab initio study of L-threonine, Spectrochim. Acta A 57 (2001) 2513–2523.
[23] X. Cao, G. Fischer, Infrared spectral, structural, and conformational studies of zwitterionic L-tryptophan, J. Phys. Chem., A 103 (1999) 9995–10003.
[24] M.T. Rosado, M.L.T.S. Duarte, R. Fausto, Vibrational spectra of acid and alkaline glycine salts, Vibr. Spectrosc. 16 (1998) 35–54.
[25] R.K. Khanna, P.J. Miller, Long wave-length phonon spectra of γ-glycine, Spectrochim. Acta A 26 (1970) 1667–1674.
[26] E. Ramachandran1, K. Baskaran, S. Natarajan, XRD, thermal, FTIR and SEM studies on gel grown γ-glycine crystals, Cryst. Res. Technol. 42 (2007) 73–77.
[27] W. Ostwald, Z. Phys. Chem. (Leipz.) 22 (1897) 289–330.
[28] R.A. van Santen, The Ostwald step rule, J. Phys. Chem. 88 (1984) 5768–5769.
[29] I. Weissbuch, V.Y. Torbeev, L. Leiserowitz, M. Lahav, Angew. Chem., Int. Ed. 44 (2005) 3226–3229.
[30] J.D. Shore, D. Perchak, Y. Shnidman, Simulations of the nucleation of AgBr from solution, J. Chem. Phys. 113 (2000) 6276–6284.
[31] R. Zhou, X. Huang, C.J. Margulis, B.J. Berne, Hydrophobic collapse in multidomain protein folding, Science 10 (2004) 1605–1609.
[32] J.L. MacCallum, M.S. Moghaddam, H.S. Chan, D.P. Tieleman, Hydrophobic association of {alpha}-helices, steric dewetting, and enthalpic barriers to protein folding, PNAS 104 (2007) 6206–6210.
[33] E.J. Sorin, Y.M. Rhee, V.S. Pande, Does water play a structural role in the folding of small nucleic acids? Biophys. J. 88 (2005) 2516–2524.